9000036

## THIE UNITED STAYTES OF ANTERIOA

TO ALL TO WHOM THESE PRESENTS SHALL COME;

### E. I. du pont de Nemours & Co.

Tahereas, there has been presented to the

### Secretary of Agriculture

AN APPLICATION REQUESTING A CERTIFICATE OF PROTECTION FOR AN ALLEGED NOVEL VARIETY OF SEXUALLY REPRODUCED PLANT, THE NAME AND DESCRIPTION OF WHICH ARE CONTAINED IN THE APPLICATION AND EXHIBITS, A COPY OF WHICH IS HEREUNTO ANNEXED AND MADE A PART HEREOF, AND THE VARIOUS REQUIREMENTS OF LAW IN SUCH CASES MADE AND PROVIDED HAVE BEEN COMPLIED WITH, AND THE TITLE THERETO IS, FROM THE RECORDS OF THE PLANT VARIETY PROTECTION OFFICE, IN THE APPLICANT(S) INDICATED IN THE SAID COPY, AND WHEREAS, UPON DUE EXAMINATION MADE, THE SAID APPLICANT(S) IS (ARE) ADJUDGED TO BE ENTITLED TO A CERTIFICATE OF PLANT VARIETY PROTECTION UNDER THE LAW.

NOW, THEREFORE, THIS CERTIFICATE OF PLANT VARIETY PROTECTION IS TO GRANT UNTO THE SAID APPLICANT(S) AND THE SUCCESSORS, HEIRS OR ASSIGNS OF THE SAID APPLICANT(S) FOR THE TERM OF eighteen years from the date of this grant, subject to the payment of the required fees and periodic replenishment of viable basic seed of the variety in a public repository as provided by LAW, the right to exclude others from selling the variety, or offering it for sale, or reproducing it, r importing it, or exporting it, or using it in producing a hybrid or different ty therefrom, to the extent provided by the Plant Variety Protection Act T. 1542, As Amended, 7 U.S.C. 2321 ET SEQ.)

SOYBEAN

'W20'

In Lestimony Whereof, I have hereunto set my hand and caused the seal of the Plant Bariety Protection Office to be affixed at the City of Washington, D.C. this 30th day of July in the year of our Lord one thousand nine hundred and ninety-three.

Secretary of Agriculture

Allest

Kanneth Hurans

Plant Variety Protection Office Agricultural Marketing Service Public reporting burden for this collection of information is estimated to average 30 minutes per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing the collection of information. Send comments regarding this burden estimate or any other aspect of this collection of information, including suggestions for reducing this burden, to Department of Agriculture, Clearance Office, DIRM, Room 404-W, Washington, D. C. 2025-00, and to the Office of Management and Budget, Paperwork Reduction Project (DMB #0581-0055), Washington, 20250

U.S DEPARTMENT OF AGRICULTUR AGRICULTURAL MARKETING SERVIC	E SE . 2000	Application is required in order to determine it a plant variety protection
APPLICATION FOR PLANT VARIETY PRO	TECTION CERTIFICATE	certificate is to be issued (7. U.S.C. 2421) Information is held confidential until certificate is issued (7. U.S.C. 2426)
NAME OF APPLICANT(S) (as if is to appear on the Certificate)	2 TEMPORARY DESIGNATION OR EXPERIMENTAL NO	3 VARIETY NAME
	EXPERIMENTAL NO	
E. I. du Pont de Nemours and Company	W20	W20
4 ADDRESS (street and no. or R.F.D. no., city, state, and ZIP)	5 PHONE (include area code)	FOR OFFICIAL USE ONLY PVPO NUMBER
1007 Market Street		
Wilmington, DE 19898	(302) 992-4927	9000036
William Indiana Colly Did 19090	(302) 332 4327	€ Date
		11/ov 221989
6 GENUS AND SPECIES NAME 7. FAMILY	NAME (Bolanical)	Time
Glycine max (L.) Merr. Legum	inoseae	
8 CROP KIND NAME (Common Name)	9 DATE OF DETERMINATION	1 E 1. 7160 -
Soybean	6-16-87	S Care
10 IF THE APPLICANT NAMED IS NOT A "PERSON," GIVE FORM OF ORGANIZATION (C		" Nov. 221989
Corporation		C Certificate Fee
11 IF INCORPORATED, GIVE STATE OF INCORPORATION	12 DATE OF INCORPORATION	- f :250,000
		E Usle 2 1993
Delaware  13 NAME AND ADDRESS OF APPLICANT REPRESENTATIVE(S), IF ANY, TO SERVE IN T	9-2-15	0 July 2 / 1 / 13
Bruce W. Morrissey	INS APPLICATION AND RECEIVE NEED IN ENG	V V
E.I. du Pont de Nemours and Company - 1	Legal Department	
Barley Mill Plaza - P17-2216	begar beparement	•
Wilmington, DE 19898	PHONE (Include area co	de) (302) 992-4927
14 CHECK APPROPRIATE BOY FOR EACH ATTACHMENT SUBMITTED (Follow INSTRU	CTIONS on reverse)	•
a Exhibit A, Origin and Breeding History of the Variety		
b. Exhibit B. Novelty Statement  c Exhibit C. Objective Description of Variety		
d Exhibit D, Additional Description of Variety	•	
Exhibit E, Statement of the Basis of Applicant's Ownership		
1 Seed Sample (2,500 wable untreated seeds) Date Seed Sample m	ailed to Plant Variety Protection Office $11-2$	1-89
g Filing and Examination Fee (\$2,150) made payable to "Treasurer of		On the Black Young
15 DOES THE APPLICANT(S) SPECIFY THAT SEED OF THIS VARIETY BE SOLD BY VAR	NO (H "NO." skip to Hem 18 below)	See section 63(a) of the claim value()
YES (# "YES." enswer dome 16 and 17 below)  16 DOES THE APPLICANT(S) SPECIFY THAT THIS VARIETY BE LIMITED AS TO	17. IF "YES" TO ITEM 16, WHICH CLASSES OF PROD	NUCTION BEYOND BREEDER SEED?
NUMBER OF GENERATIONS?		STERED CERTIFIED
LI YES LI NO	FOUNDATION REGI	STENED CENTRED
18 DID THE APPLICANT(S) PREVIOUSLY FILE FOR PROTECTION OF THE VARIETY IN T	HE U S ?	
VES # "YES." through Plant Variety Protection Act	nt Act Give date 9-22-88 )	
□ No	<del></del>	
19 HAS THE VARIETY BEEN RELEASED, USED, OFFERED FOR SALE, OR MARKETED	IN THE U.S. OR OTHER COUNTRIES?	
tak a <u>la</u>		
YES (II "YES," give names of countries and dates)  NO		
20 The applicant(s) declare(s) that a viable sample of basic seeds of the request in accordance with such regulations as may be applicable.		
The undersigned applicant(s) is (are) the owner(s) of this sexually uniform, and stable as required in section 41, and is entitled to prote	ection under the provisions of section 42 of th	vets) that the variety is distinct, e Plant Variety Protection Act.
Applicant(s) is (are) informed that false representation herein can je	opardize protection and result in penalties.	
SIGNATURE OF APPLICANT (Opener(LI)	CAPACITY OR TITLE	DATE
Wayeld at Haer	Connetary Batant Bered	11-21-89
18/10-03-00	Secretary Patent Board	DATE
SIGNETURE OF APPLICANT (Owner(s))	On north on the	

### 14A. Exhibit A - Origin and Breeding History

W20 soybean [Glycine max (L.) Merr.] is a true-breeding mutant of the variety 'Williams' developed by the Agricultural Products Department of E. I. du Pont de Nemours and Company. PVP was sought in 1989 because of it's unusually high resistance to certain sulfonylurea herbicides.

### 14.A.1 AND 14.A.2:

W20 originated as a single plant selection from an M2 population of Williams treated with a normally lethal concentration of chlorsulfuron (Sebastian et al., 1989). W20 survived the chlorsulfuron treatment and was allowed to reach maturity. M3 seed of W20 was rescreened and found to be uniformily resistant to chlorsulfuron. M4 seed of the W20 line was tested and found to be resistant to treatments of other sulfonylureas (including Harmony®, Express®, and chlorimuron ethyl) that were highly injurious to wild type soybeans (Sebastian et al., 1989).

### 14.A.3

W20 appears to be stable and uniform through five generations of selfing. No noticeable variants were observed.

### 14.A.4.

M3 family uniformity was demonstrated by showing that all 30 seeds from a single M2 plant were resistant. Genetic studies concluded that sulfonylurea resistance in W20 is inherited as a single semidominant allele named Als1. Biochemical studies indicate that Als1 confers resistance to sulfonylureas at the level of acetolactate synthase, the herbicidal site of action (Sebastian et al, 1989).

REFERENCE: S. Sebastian et al, Crop Science 29, 1403-1408 (1989) (attached).



## U.S. DEPARTMENT OF AGRICULTURE PLANT VARIETY PROTECTION OFFICE

PVP APPLICATION NO. 9000036 EXAMINER: JEFFREY L.STRACHAN

TITLE: 'W20' SOYBEAN

FILING DATE: NOVEMBER 22, 1989 WILMINGTON, DELAWARE 19898

OUR REFERENCE: BB-1008 (PVP) DATE: APRIL 15, 1992

Honorable Secretary of Agriculture Beltsville, MD 20705-2351 Sir:

#### REQUEST FOR RECONSIDERATION

In response to the Communication of November 15, 1991,
Applicant offers the following comments and information, and
respectfully requests reconsideration of this application.
Applicant notes that time period for response was set to run on
May 16, 1992.

#### REMARKS

To summarize the current status of the application, Examiner Strachan has issued a statement of findings after an initial examination following an update of the soybean database. It has been found that Applicant's Novelty Statement (Exhibit B) requires revision, and the Examiner has outlined three general ways in which Applicant might prove novelty. Further, it has been suggested that Applicant could establish novelty through a suitable comparison to the variety "Govan".

Applicant is highly appreciative of the Examiner's review and suggestion. Applicant believes that path (3) - "choose the variety believed to be the most similar to the application variety and explain how it differs from it" - is in fact the appropriate means to establish novelty. Applicant believes, however, that the most similar variety is "Williams" with "William 82" an exceedingly close second choice. Applicant will therefore demonstrate novelty by extensive comparison to these two varieties of soybean, and at the same time provide evidence of the resistance of "W20" to sulfonylurea herbicidal compounds.

The "W20" variety was derived from mutagenesis of "Williams", it is therefore a near-isogenic line of Williams. Hence Williams is the most closely related soybean line to W20 and should be used for comparison purposes in the examination of the plant variety protection registration application. "Williams 82" is also a closely-related near-isogenic line for comparison to W20. Williams 82 was derived by backcrossing the  $\operatorname{Rps1}^k$  gene from Kingwa into Williams. The  $\operatorname{Rps1}^k$  gene confers resistance to Phytophtora megasperma f.sp. glycinea. cycles of backcrossing were performed, Williams 82 should be approximately 99% similar to both Williams and W20 in terms of genetic background. Williams 82 and Williams are indistinguishable in terms of reaction to sulfonylurea herbicides and both lack the high degree of resistance found in W20. The preparation of "W20" by mutational breeding from "Williams" is detailed in U.S. Patent 5,084,082 columns 16 (lines 18-55) and 17 (lines 26-54 and TABLE II). A copy is attached for the Examiner's convenience.

Although "Williams" and "Williams 82" are the most similar varieties to "W20" there are nevertheless major differences between these varieties and "W20". Most significant is the trait of sulfonylurea herbicide resistance. Applicant has established this difference through preemergent and postemergent applications of a number of sulfonylurea herbicide compounds in comparative tests of "W20" vs. "Williams" and "W20" vs. "Williams 82". In a preemergent comparative test of herbicidal resistance, twenty seeds of each of "Williams" and "W20" were soaked for 14 hours in a solution of 1 ppm chlorsulfuron (2chloro-N-[(4-methoxy-6-methyl-1,3,5-triazin-2-yl)aminocarbonyl] benzenesulfonamide). As a control, twenty additional seeds of each line were soaked for 14 hours in distilled water. end of this period, the seeds were completely imbibed with either chlorsulfuron or distilled water. Imbibed seeds were then planted in pots (4" x 4") containing a standard peat-based potting soil (Metro-Mix 350) at a density of 10 seeds per pot. This gave a total of 2 pots for each of 6 treatments (3 genotypes x 2 herbicide treatments). Pots were placed in the greenhouse and watered daily. At 7 days after treatment, it was observed that control plants were fairly uniform in appearance and had reached the first unifoliolate stage. Of the plants

treated with chlorsulfuron, only the "W20" plants were forming leaves. The health of the "W20" plants was not visibly different than that of the controls. However, chlorsulfuron-treated plants of "Williams" had emerged and opened cotyledons but did not develop true leaves. Since plant-to-plant variation within pots was negligible, pots were thinned back to 4 to 5 plants per pot to permit further development without crowding. At 14 days after the seed soak treatment, controls and chlorsulfuron-treated "W20" plants were still developing normally and were at the second trifoliolate stage. A visual rating of herbicide injury of each pot was given using the control plants as the standard of 0% injury. Injury was based on the extent of true leaf development past the stage of cotyledon expansion.

The difference between "W20" and the standard soybean cultivar Williams was very dramatic (see Table 1 below). Chlorsulfuron-treated "W20" plants were indistinguishable from controls (0% injury). Chlorsulfuron-treated "Williams" plants had only vestigial leaves that were completely arrested in development; these plants had still not developed past the point of cotyledon opening (100% injury). Statistical analysis of the data was practically meaningless since lines were either completely inhibited or completely unaffected by the herbicide treatment.

TABLE 1: INJURY OF CONTROL AND CHLORSULFURON-TREATED SOYBEAN PLANTS AT 14 DAYS AFTER SEED-SOAK TREATMENT

VARIETY	CHLORSULFURON RATE (PPM)	POT	%INJURY (mean of 4-5 plants)
WILLIAMS	0	1 2	0
W20	0	1 2	0 0
WILLIAMS	1	1 2	100 100
W20	1	1 2	0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0

A postemergent comparison of "W20" and "Williams" has also been conducted using another sulfonylurea herbicide, thifensulfuron methyl (methyl 3-[[[(4-methoxy-6-methyl-1,3,5triazin-2-yl]amino]carbonyl]-amino]sulfonyl]-2-thiophenecarboxylate). Four plastic tubs (18x11x5 inches) were filled with a sandy loam-peat-vermiculite mix and rows of W20 and Williams soybeans were planted lengthwise in each. were also planted with rows of pigweed, velvetleaf and lambsquarters. The other two were planted with cocklebur and morningglory (while these weed species were included in the test, results will not be reported herein except to say that weed control on all species was good to excellent at both rates tested). Plantings were made April 1, 1991 and when the soybeans reached the first trifoliate leaf stage (April 15, 1991), treatments were applied as a postemergence spray at rates of 4 and 17 g/ha to the soybeans and weeds. Surfactant X-77 was included at 0.25%. A tub of each was planted and left untreated for use as a control. The results are presented in Table 2 below:

TABLE 2. RESPONSE OF W20 AND WILLIAMS SOYBEANS TO 4 AND 17 g/ha THIFENSULFURON METHYL POSTEMERGENCE

•		Percent injury			
VARIETY	CONTROL	<u>4 g/ha</u>	<u>17 g/ha</u>		
Williams	0	0	60-70		
W20	0	0	0		

Thifensulfuron methyl had little or no effect on either Williams or W20 soybeans at 4 g/ha 2-3 weeks after treatment. However, at 17 g/ha, Williams soybeans showed unacceptable injury (60-70%). Soybean growing points were killed and new growth had to emerge from the leaf axils. In contrast, W20 soybeans showed no injury; after 2-3 weeks growing points were unaffected. These data demonstrate that even with thifensulfuron methyl, a compound used commercially for weed control in soybeans, W20 soybeans are uninjured at rates that cause severe injury to wild-type soybeans.

Detailed comparisons of "W20" and "Williams 82" have also been conducted to distinguish the two varieties. These data are incorporated in U.S. Patent 5,084,082 (attached). The

Examiner's attention is directed to Table VI (column 34), Table VII (column 36), Table VII-A (column 37) and Table IX (column 39). These data clearly establish that "W20" can withstand both preemergent and postemergent application of a number of commercial sulfonylurea herbicidal compounds. Side-by-side comparison shows that "Williams 82" suffers agronomic injury while "W20" is generally not significantly injured.

With regard to agronomic traits, growout of "W20" and "Williams" at Stine Labs, Newark, Delaware in the summer of 1989 provide the following comparisons:

<u> Trait                                    </u>	<u>W20</u>	<u>Williams</u>
Height	2-3 inches shorter	
Maturity	2.5 days earlier	
Lodging	10% more lodging	:

Yield

5% greater

Applicant has amended the Novelty Statement of Exhibit B through detailed comparisons of "W20" and the most similar soybean varieties, "Williams" and "Williams 82". Applicant believes it has established the protectability of soybean variety "W20", and the early issuance of a notice of allowance of plant variety protection is solicited.

Respectfully submitted,

Bruce W. Morrissey
Attorney/Applicant's

Attorney/Applicant's
Representative
Telephone: (302) 992-49

Telephone: (302) 992-4927

Attachment 21/dmj

EXHIBIT C (Saybeen)

U.S. DEPARTMENT OF AGRICULTURE AGRICULTURAL MARKETING SERVICE LIVESTOCK, MEAT, GRAIN & SEED DIVISION PLANT VARIETY PROTECTION OFFICE BELTSVILLE, MARYLAND 207.05

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FORM ( MGS-470-57 (2-82)

# OBJECTIVE DESCRIPTION OF VARIETY SOYBEAN (Glycine max L.)

NAME OF APPLICANT(S)	TEMPORARY DESIGNA	TION VARIETY NAME
E. I. du Pont de Nemours and Company	W20	w20
ADDRESS (Street and No., or R.F.D. No., City, State, and Zip C		FOR OFFICIAL USE ONLY
1007 Market Street	·	900036
Wilmington, DE 19898		"1.4 b.1 When the number of significant digits
Choose the appropriate response which characterizes the in your answer is fewer than the number of boxes provide	variety in the features desi ed, place a zero in the first	box when number is 9 or less (e.g., 0 9).
1. SEED SHAPE:		
1 = Spherical (L/W, L/T, and T/W ratios = < 1.2) 3 = Elongate (L/T ratio > 1.2; T/W = < 1.2)		attened (L/W ratio > 1.2; L/T ratio = < 1.2) attened (L/T ratio > 1.2; T/W > 1.2)
2. SEED COAT COLOR: (Mature Seed)		
1 = Yellow 2 = Green 3 = Brown	4 × Black 5	= Other (Specify)
3. SEED COAT LUSTER: (Mature Hand Shelled Seed)		
1 = Duff ('Corsoy 79'; 'Braxton') 2 = Shiny ('N	ebsoy'; 'Gasoy 17')	
4. SEED SIZE: (Mature Seed)		
1 8 Grams per 100 seeds (this is the se	eed size in one er	nvironment only)
5. HILUM COLOR: (Meture Seed) RWS 8-11-92	A A A A A A A A A A A A A A A A A A A	
6 X 1 - Buff 2 = Yellow 3 - Brown	4 = Gray 5 = Impl	erfect Black 8 = Black 7 = Other (Specify)
6. COTYLEDON COLOR: (Mature Seed)		
1 - Yellow 2 = Green		
7. SEED PROTEIN PEROXIDASE ACTIVITY:		
1 - Low 2 = High		
8. SEED PROTEIN ELECTROPHORETIC BAND:		
1 = Type A (SP1 <sup>a</sup> ) 2 = Type B (SP	1 <sup>b</sup> )	
9, HYPOCOTYL COLOR:	***************************************	•••
	: 71'}	ryledons ('Woodworth'; 'Tracy')
10. LEAFLET SHAPE:		
3 1 = Lanceolate 2 = Ovol 3 = C	)vate 4 = Other (Spe	ecity)

5. Shii

Acres Edward

n DISEA	SE REACTION: (	Enter 0 = Not Tested; 1 = Susceptible; 2 = Res	sistent) (Continued)	9000036
	GAL DISEASES:			
		light (Diaporthe phaseotorum var; sojae)		
		n (Cercospora kikuchil)		
		ot Rot (Rhizoctonia solani)		
ركي		ot (Phytophthora megasperma var. sojae)		
1	Race 1	1 Race 2 1 Race 3 1	Race 5	1 Race 6 1 Race 7
1	Race 8	1 Race 9 1 Other (Specify) sh	ou <u>ld be same as W</u> i	lliams but not tested
VIR	AL DISEASES:			
0	Bud Blight (Tot	oscca Ringspot Virus)	•	
0	Yellow Mosaic	(Bean Yellow Mosaic Virus)		
0	Cowpus Mossic	(Cowpes Chlorotic Virus)		
0	Pod Mottle (Be	an Pod Mottle Virus)		
0	Seed Mottle (Se	ybean Mossic Virus)		
NE	, MATODE DISEAS	ES:		
		jematode (Heterodera glycines)		
0:	Race 1		Race 4 Other (Sp	ecify) should be same as William but not tested
0	Lance Nematod	de (Hapialaimus Colombus)		
0	Southern Root	Knot Nematode (Maloidogyna incognita)		
0	Northern Root	Knot Nematode (Meloidogyne Hapla)		
	Peanut Root K	ngt Nematode (Melaidogyne arenaria)	•	•
	Reniform Nem	atode (Rotylenchulus reniformis)		
	J	ASE NOT ON FORM (Operally /	should tested	be same as Williams but not
ــــــــــــــــــــــــــــــــــــــ				
0, PHYS	IOLOGICAL RES	PONSES: (Enter 0 = Not Tested; 1 = Suscept	ible; 2 = Resistant)	
0	Iron Chlorosis	on Calcereous Soil	·	
	Other (Specify			
i, INSE	TREACTION:	Enter 0 * Not Tested; 1 = Susceptible; 2 = Re	sistant)	
0	Mexican Bean	Beetle (Epilachna varivestis)		
0	Porato Leaf He	opner (Emonasca fabat)		
	Other (Specify	1		
	~ 	RIETY MOST CLOSELY RESEMBLES THA		
	ARACTER	NAME OF VARIETY	CHARACTER	NAME OF VARIETY
Plant		Williams	Seed Coat Luster	Williams
Leaf 3		Williams	Caarl Siza	Williams
Lvaf (	Color	Williams	Seed Shape	Williams
Leaf \$	Size	Williams	Seedling Pigmentation	Williams //
				10

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FORM LMGS-470-57 (2-82)

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A STATE OF THE PARTY OF THE PAR	AD CTANDARD VARIETY: Paired Companion, Fire
	All STATEMINE THE

YARISTY	NO OF DAYS MATURITY	PLANT LODGING SCORE	CM PLANT HEIGHT	I FAFI ET SIZE		SEED CONTENT		SEED SIZE G/100	NO. \$EED9/ POD
				CM Width	CM Length	% Protein	% ሆዘ	SEEuc	
W20 Submitted	128.1	59%	95.4						
illiams Name of Similar Variety	131.4	55%	98.1						

## PUBLICATIONS USEFUL AS REFERENCE AIDS FOR COMPLETING THIS FORM:

- 1. Caldwell, B.E., ed. 1973. Soybeans: Improvement, Production, and Uses. Amer, Soc. Agron. Monograph No. 16.
- 2. Buttery, B.R. and R.I. Buzzell. 1968. Peroxidase activity in seeds of soybean varieties. Crop Sci., 8: 722-725.
- 3. Hymowitz, T. 1973. Electrophoretic analysis of SBTI-A2 in the USDA soybean garmplasm collection. Crop Sci., 13: 420-421.
- 4. Payne, R.C. and L.F. Morris. 1976. Differentiation of soybean cultivars by seedling pigmentation patterns. J. Seed Technol. 1: 1-19.

### 14E. Exhibit E - Basis of Applicant's Ownership

The soybean variety, W20, for which Plant Variety Protection is hereby sought was developed by Dr. Scott A. Sebastian, an employee of E. I. du Pont de Nemours and Company. By agreement between Dr. Sebastian and Du Pont (Dr. Sebastian's Employment Contract) all rights to any invention, discovery, development, patent, or other intellectual property made by Dr. Sebastian while employed by Du Pont have been assigned to Du Pont with no rights of any kind retained by Dr. Sebastian.